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(54) Title: ENZYMATIC COMPOSITION

(57) Abstract

Enzymatic compositions with improved storage stability of the enzymes contained therein are obtained by including an enzyme stabiliser, preferably by way of a particular process.

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ENZYMATIC COMPOSITION

Technical field

The present invention relates to a enzymatic composition with improved storage stability of the enzymes contained therein.

Background & Prior art

It is well known in the art that enzymes can lose their activity with time when included in an aqueous liquid detergent composition, and various proposals have already been made to retard that loss of activity by including in such compositions an enzyme-stabilizing system. Various enzyme stabilisers have been suggested in the art for inclusion in liquid detergent compositions, e.g. polyols (e.g. glycerol), borax (preferably in combination with glycerol), calcium ions, alcohols, low molecular weight carboxylates (formate, acetate, propionate, etc.) and polymers (e.g. poly-vinyl-pyrollidone).

We have surprisingly found that inclusion of a certain class of compounds in such aqueous enzymatic liquid detergent compositions retards the loss of enzyme activity.

Statement of the Invention

We have found that enzyme stability can be improved by using the class of compounds that embraces the group of lignin compounds.

Therefore, the present invention relates to an enzymatic detergent composition with an improved storage stability of enzyme material contained therein, the improved storage stability being obtained by the inclusion in the composition of a lignin compound.

Description of the Invention

Lignin compounds are mixtures of components and is usually referred to as a polymer which contains, amongst others, phenylpropane units. Lignin compounds can be prepared from the chemical pulping of hard- and softwoods. Lignin compounds have been found to be very effective compounds according to the present invention. There are various lignin compounds which are preferred enzyme stabilisers according to the invention, including Lignosulphonates, Kraft lignins and Oxylignins. All these compounds are considered lignin compounds. These compounds may be prepared from Lignin by various ways, including:

1) treatment with hot (acid) solution of calcium bisulphite which generates Lignosulphonates. The Lignin undergoes a sulphonation and a hydrolysation process under the influence of sulphite.

2) treatment with hot alkaline (pH 13-14) solution of sodium sulphate generates Kraft Lignins, which may subsequently be modified in various ways, e.g. sulphonated, methylated, carboxylated and/or fractionated.

3) reducing the sulphur content of lignosulphonate raw material and optionally applying condensation, cleavage and/or rearrangement, to reduce the number of sulphonic and methoxyl groups and to increase the number of functional phenolic, hydroxyl and carboxylic groups generates Oxylignins.

Further variations to Lignin or any of its derivatives may be made by varying the kind of cation (Na^+, K^+, Ca^{2+}, Mg^{2+}, $NH4^+$, the degree of sulphonation and/or the average molecular size.

Examples of lignin derivatives that have been found useful are Borresperse NA, Borresperse CA, Kelig FS, Maracarb N-1, Marasperse N-22, Marasperse N-3, Norlig BD, Norlig 415, Ufoxane 2, Ufoxane 3A, Maracell 3A, Vanisperse CB, Ultrazine NA, Ultrazine CA (all ex Borregaard) and lignosulphonates ex Aldrich and ex Sigma as well as ex a number of pharmaceutical companies.

We have found that inclusion of lignin compounds significantly retards the enzyme deactivation, and most surprisingly, lignin compounds are effective as stabiliser at low concentration. Consequently, lignin compounds are included in effective amounts in the composition, in particular in the range of 0.0001 to 10%, preferably 0.001 to 5%, more preferably at least 0.01 and more preferably at most 3% by weight of the composition.

Although the weight ratio between lignin compound and enzyme (as defined as the weight of the active enzyme protein material, which does not include any additives that for example may be present in the enzyme preparations as supplied by the enzyme manufacturers) may be varied widely, as long as the enzyme is effectively stabilised, a weight ratio between 1000:1 and 1:10 has been found to be preferred, more preferably lower than 500:1, most preferably lower than 100:1, in particular lower than 50:1, whereas it is more preferred to have a weight ratio of higher than 1:5, most preferably higher than 1:3, in particular 1:2, more in particular 1:1.

Preferably, the molar ratio between lignin compound and enzyme is from 0.1 to 10,000, more preferably at least 1 and at most 5,000, most preferably at least 2.

It will be understood that presence of other enzyme stabilising systems is not excluded in compositions according to the invention.

Lignin compounds have been described in the art for several applications.

GB-A-1,403,257 discloses use of lignin in enzyme preparations which may be included in solid compositions. The enzyme preparations are purified by precipitating protease or α-amylase with a tanning agent or lignin, whereafter the solid enzyme preparation is filtered off.

DE 23 54 791 discloses the use of lignosulfonates as coating material for enzyme granules for use in powdered compositions.

DD 237,522 discloses a process for cleaning an enzyme concentrated containing protease and/or by α-amylase by precipitating undesired polution.

Use of lignin preparations to inhibit enzyme activity at low pH in the human stomach is discussed in ZA 6803394 and in EUR J Pharmacol 41 (2) 1977 p 235-238; coden: EJPHAZ ISSN: 0014-2999 [EMW].

WO 94/19529 discloses a process for providing localized variation in the colour density of fabrics by using a cellulase enzyme and a polymeric agent.

The invention further relates to a liquid enzymatic composition comprising from one or more enzymes and one or more enzyme stabilisers, characterised in that the stabiliser comprises a water-soluble, cross-linked polymer

containing sulphonate-groups, preferably containing benzene units and more preferably containing phenylpropane units.

The enzymatic composition of the present invention contains as essential ingredients one or more enzymes, preferably at least including a proteolytic enzyme.

The enzymes that may be used in the present invention are proteases, amylases, lipases, cellulases and mixtures of one or more of these enzymes. Proteases are preferred enzymes for use in the present invention, as we have seen the best results with protease stabilisation.

Depending on the type of composition (i.e. diluted or concentrated enzyme composition) and, of course, whether the enzyme type is present at all, the enzymes preferably provide a proteolytic activity between 0.1 and 50 GU/mg, a lipolic activity between 0.005-100 LU/mg and an amylotic activity between 10^3 to 10^7 MU/kg, wherein GU, LU and MU units are well known in the art and have e.g. been defined in lines 8-14 of column 3 and lines 8-12 and 21-24 of column 4 of US 5,112,518.

Depending on the composition type, the level of active enzyme protein will be higher (up to 10%, preferably up to 5% by weight for concentrated enzyme preparations, e.g. as supplied by enzyme manufacuturers) or lower (up to 3%, preferably up to 1.0%, although levels up to 0.5% or up to 0.1% or even as low as up to 0.05% are also suitable for more dilute systems, e.g. commercial liquid detergent compositions in which the concentrated enzyme preparations are used during production). The active enzyme protein level may be as low as 0.0001%, preferably at least 0.01% by weight of the composition. Again in more concentrated

enzyme preparations, the lower level will be higher, e.g. at least 0.5% by weight.

We have further found that combinations of enzymes (especially when they include proteases) may be stabilised by using the invention. As compared to the composition without the stabiliser, they show strongly improved stability overall.

Preferably, detergent-active component is included and may be either soap, anionic, nonionic, cationic or zwitterionic detergents and mixtures of one or more of these detergent-active components. Preferably, nonionic detergent is used, either as such or in admixture with a anionic detergent-active component. Usually, the total amount of detergent-active component(s) ranges from 5% to 70%, preferably from 10 to 40% by weight of the total composition.

Preferably, compositions according to the invention have an ionic strength and contain electrolyte material. Preferably, electrolyte material is selected from the group consisting of phosphate, phosphonate, borate, carboxylates (e.g. citrate, NTA and succinate such as C12 alk(en)ylsuccinate), carbonate, sulphate and chloride. Preferably, the electrolyte material is present at a level of at least 1%, more preferably at least 2%, most preferably at least 3%, in particular at least 5%, e.g. at least 10% by weight of the composition. Suitable levels are at least 15% by weight of the composition. Preferably, the composition comprises less than 25% by weight of electrolyte material.

The composition may furthermore contain other optional ingredients such as perfumes, colouring materials, soil-suspending agents, other enzyme-stabilising agents,

builder, bleaching agents, bleach precursors, hydrotropes, solvents, suspending agents, suds suppressors, polymers (e.g. for oily soil or particulate soil removal or as anti-due transfer agent), fluorescers, etc.

The enzymatic composition may be in the powdered form, but is preferably in the liquid form. The composition may be an isotropic or a structured liquid. Structured liquids (i.e. containing lamellar droplets of surfactant material) are the most preferred liquids.

Preferably, liquids according to the present invention are prepared by mixing an enzyme preparation and one or more enzyme stabilisers, wherein the enzyme stabiliser comprises lignin compounds.
Preferably, the pH of the liquid formulations according to the present invention is higher than 4, more preferably higher than 5, most preferably higher than 5.5 and preferably lower than 11, more preferably lower than 10, most preferably 9.0 or lower.

To improve the enzyme stability even further, the lignin compound is preferably brought in contact with the enzyme in a form in which the lignin is at least partially dissolved. This may be done in various ways, including chosing a certain order of addition that results in this effect. A premix of enzyme and lignin can be made which is then mixed with the other ingredients or lignin is added in the form of a solution, preferably in the form of a solution in a solvent, e.g. selected from alcohols and/or water. Examples of suitable solvent systems are water and a 25% propyleneglycol solution.

The invention will now be illustrated by way the following non-limiting examples.

EXAMPLES

Example 1

The following formulation 1 was prepared:

Ingredients	% by weight
LAS (Na salt)	23
Nonionic*	10
Citrate (Na salt)	17
Polymer material**	1.0
Savinase 16.0L (ex NOVO)	0.38%
Minors	0.25
Water	to 100%

* Nonionic is an ethoxylated alcohol.

** (as 100%) Polymer A11 as desecrbed in EP 346,995.

The protease stability at 37°C was measured in the presence of various levels of sodium ligno-sulphonate. The following results were obtained after 10 days.

% by weight Ultrazine NA * ***	% residual activity
0	25
0.005	52
0.010	63
0.015	68
0.025	80
0.050	89
0.100	82

*** a sodium lignosulphonate, ex Borregaard, added on top of formulation in powdered form.

It can be clearly seen that the lignin compound has good enzyme stabilising properties, even at very low concentrations.

Example 2

Lipolase® (100L, ex NOVO) was added to formulation 1 of Example 1 at a level of 0.2% and the lipase activity was determined after 10 days storage at 37°C.

% by weight Ultrazine NA® ***	% residual activity
-	3
0.005	3
0.010	5
0.015	5
0.025	38
0.050	50
0.100	80#

*** a sodium lignosulphonate, ex Borregaard, added on top of formulation in powdered form.

extrapolated from 7 days' stability data.

It can be clearly seen that the lignin compound has good lipolase stabilising properties in the presence of protease.

Example 3

The following liquid formulation 2 was prepared by neutralising a premix of the detergent active material, mixing in the builder material and the minors. Enzyme

material was added as last ingredient. Stabiliser (if any) was post-dosed.

Ingredients	% by weight
Anionic	16.5
Nonionic	4.5
Oleic acid	4.5
Citric acid	8.2
Zeolite	15.0
KOH	10.3
Polymer*	1.0
Protease**	0.38
Lipase ***	0.2
Minors	0.9
Water	to 100

pH liquid 8.5

* Polymer A11 of EP 346995
** Protease is Savinase 16.0L (ex Novo)
*** Lipase is Lipolase 100L (ex Novo)

The enzymatic activites in the liquid after 28 days of storage at 37°C was as follows when Ultrazine NA was added in the form of a solution in 25% propyleneglycol solution:

% by Ultrazine NA	% Residual protease act. (no lipase)	% Residual protease act. (with lipase)	% Residual lipase act. (with prot.)
0	30	24	0
0.025	58	63	22
0.05	75	73	43
0.1	79	80	53

The enzymatic activites in the liquid after 28 days of storage at 37°C was as follows when Ultrazine NA was added in solid form:

% by Ultrazine NA	% Residual protease act. (no lipase)	% Residual prot. act. (with lipase)	% Residual lipase act. (with prot.)
0	30	24	0
0.025	36	35	7
0.05	48	49	15
0.1	52	58	28

It can be clearly seen that the lignin compound has good protease and lipase stabilising properties, even at very low concentrations.
Addition of the Ultrazine NA in soluble form results in even better enzyme stability.

Example 4

The formulation of Example 1 was prepared. Stabiliser (if any) was post dosed. The enzymatic activites in the liquid after 14 days of storage at 37°C was as follows when Ultrazine NA was added in a solution in 25% propyleneglycol in water:

% by weight Ultrazine NA	% Residual protease activity (with lipase)	% Residual lipase activity (with protease
0	13	4
0.025	57	25
0.05	67	53
0.1	70	63

The enzymatic activites in the liquid after 14 days of storage at 37°C was as follows when ultrazine NA was added in solid form:

% by weight Ultrazine NA	% Residual protease activity (with lipase)	% Residual lipase activity (with protease
0	13	4
0.025	50	24
0.05	62	53
0.1	66	60

It can be clearly seen that the lignin compound has good protease and lipolase stabilising properties, even at very low concentrations. Addition of the Ultrazine NA in soluble form results in even better enzyme stability.

Example 5

The formulation of Example 3 was prepared. Various lignin compounds were added at a level of 0.1% by weight and the following stabilisation results were obtained, expressed as residual activity (in % of original activity) relative to the blanc (i.e. delta value).

	Delta % residual act.	
Lignin compound	Lipase	Protease
Marasperse N-22 (ex Borregaard)	37	47
Marasperse N-3 (ex Borregaard)	29	29
Marasperse AG (ex Borregaard)	26	29
Maracell (ex Borregaard)	46	50
Maracarb (ex Borregaard)	45	40
Norlig 612 (ex Borregaard)	34	43
Norlig (ex Borregaard)	38	45
Ultrazine NA (ex Borregaard)	48	57
Borresperse CA (ex Borregaard)	36	44
Borresperse NA	39	38
Ultrazine CA (ex Borregaard)	44	50
Ufoxane 2 (ex Borregaard)	30	29
Ufoxane 3A (ex Borregaard)	38	35
Na-lignosulphonate (ex Aldrich)	39	44

All lignins show lipase and protease stabilising effects.

CLAIMS

1. An enzymatic composition comprising from one or more enzymes and one or more enzyme stabilisers, wherein the stabiliser comprises a lignin compound and wherein the composition is liquid.

2. Composition according to claim 1, wherein the enzyme is selected from proteases, amylases, lipases and mixtures thereof.

3. Composition according to claims 1-2 comprising from 0.0001 to 10% by weight of a lignin compound.

4. Composition according to claims 1-3, wherein the enzyme comprises a protease.

5. Composition according to claims 1-4, further comprising from 1 to 25 % by weight of electrolyte material.

6. Composition according to claims 1-5, further comprising from 5 to 70% by weight of surfactant material.

7. A liquid enzymatic composition comprising from one or more enzymes and one or more enzyme stabilisers, characterised in that the stabiliser comprises a water-soluble, cross-linked polymer containing sulphonate-groups.

8. Process of preparing an enzymatic composition by mixing an enzyme preparation and one or more enzyme stabilisers, wherein the enzyme stabiliser comprises lignin compounds.

9. Process according to claim 8, where in the lignin compound is brought in contact with the enzyme in a form in which the lignin is at least partially dissolved.

10. Process of mixing a lignin compound with an aqueous enzyme preparation or a non-aqueous surfactant containing enzyme preparation.

INTERNATIONAL SEARCH REPORT

Interna l Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C11D3/386 C11D1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C11D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 19529 A (VOLLMOND THOMAS ;LUND HENRIK (DK); TOFT ANNETTE HANNE) 1 September 1994 cited in the application see claims; example 5 ---	1
A	DE 23 54 791 A (UNILEVER NV) 9 May 1974 cited in the application see claims; example 20 ---	1
A	DD 237 522 A (INSTITUT FÜR TECHNISCHE MIKROBIOLOGIE) 16 July 1986 cited in the application see page 1; examples ---	1
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[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 1 403 257 A (KALI CHEMIE AG) 28 August 1975 cited in the application see claims	1
X	EP 0 626 445 A (ALLIED COLLOIDS LTD.) 30 November 1994 see page 3, column 3, line 21 - page 4, column 5, line 17; claims 1,3,5	7

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INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 96/02586

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9419529	01-09-94	AU-A- 6139394	14-09-94
DE-A-2354791	09-05-74	GB-A- 1435905	19-05-76
		AT-B- 336529	10-05-77
		BE-A- 806856	30-04-74
		CA-A- 994266	03-08-76
		CH-A- 584234	31-01-77
		FR-A- 2205532	31-05-74
		NL-A- 7315113	07-05-74
		SE-B- 411769	04-02-80
		US-A- 4009076	22-02-77
DD-A-237522		NONE	
GB-A-1403257	28-08-75	DE-A- 2143945	08-03-73
		AT-B- 321230	25-03-75
		BE-A- 787686	18-12-72
		CH-A- 575962	31-05-76
		FR-A- 2154447	11-05-73
		LU-A- 65913	15-01-73
		NL-A- 7211170	06-03-73
		SE-A,B,C 375322	
EP-A-626445	30-11-94	AU-B- 637323	27-05-93
		AU-A- 4022289	08-03-90
		AU-B- 637577	03-06-93
		AU-A- 4022689	01-03-90
		AU-B- 634719	04-03-93
		AU-A- 4022789	01-03-90
		CA-A- 1336694	15-08-95
		DE-D- 68910925	05-01-94
		DE-T- 68910925	23-02-95
		DE-D- 68919942	26-01-95
		DE-T- 68919942	27-07-95
		DE-D- 68921266	30-03-95
		DE-T- 68921266	22-06-95
		DK-A- 2796	12-01-96
		DK-B- 171065	13-05-96
		DK-B- 171073	28-05-96
		DK-B- 171054	06-05-96

INTERNATIONAL SEARCH REPORT

Interna ! Application No
PCT/EP 96/02586

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-626445		EP-A- 0361677	04-04-90
		EP-A- 0356239	28-02-90
		EP-A- 0356240	28-02-90
		FI-B- 92712	15-09-94
		FI-B- 96036	15-01-96
		JP-A- 2102298	13-04-90
		JP-A- 2111718	24-04-90
		JP-A- 2150280	08-06-90
		NO-B- 175601	25-07-94
		NO-B- 176278	28-11-94
		NO-B- 176248	21-11-94
		US-A- 5460817	24-10-95
		US-A- 5035900	30-07-91
		US-A- 5324445	28-06-94
		US-A- 5492646	20-02-96